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X-Arrestin: a new retinal arrestin mapping to the X chromosome

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We have been using a differential cDNA cloning approach to isolate human retina-specific and retina-enriched genes [1]. A 1,314 bp cDNA was isolated by this approach, representing a highly retina-specific message encoding a 388 amino acid protein showing 58%, 50%, and 49% homology to bovine β-arrestin, and bovine and human retinal arrestin (S-antigen), respectively. Chromosomal mapping localized this new arrestin gene to the proximal long arm of the X chromosome, hence it was named X-arrestin. In situ hybridization demonstrated its expression in the inner and outer segments and the inner plexiform regions of the retina.

Arrestin; β-Arrestin; Retina; cDNA; X chromosome; Hybridization, in situ

1. INTRODUCTION

Isolation and characterization of retinal genes, such as rhodopsin [2], peripherin [3], cGMP-phosphodiesterase (the β subunit of which is the gene involved in a mouse model of retinal degeneration, the rd mouse) [4], arrestin (S-antigen) [5], α-transducin [6], phosducin [7], recoverin [1], cellular retinaldehyde binding protein [8], and interphotoreceptor retinol binding protein [9], have contributed greatly towards understanding retinal biology. A candidate gene approach using these genes (rhodopsin and peripherin) has led to the identification of the causative gene in some cases of autosomal dominant retinitis pigmentosa [10–12]. In an effort to further understand the biology of the human retina in normal and diseased states, we have been using a differential cDNA cloning approach to isolate human retina-specific and retina-enriched genes. This approach has already resulted in the isolation of the human recoverin cDNA and gene [1]. We report here the isolation of another retina-specific gene, arrestin, by this approach.

Retinal arrestin or S-antigen is a phototransduction protein found abundantly in the photoreceptors and thought to play a role in inactivation of light-activated rhodopsin [13,14]. S-Antigen is thought to quench the phototransduction cascade by binding to light-activated, phosphorylated rhodopsin, thereby blocking its interaction with transducin. S-Antigen is well conserved among species, and homologues have been isolated from cow, rat, human, and fruit fly [5,15–18]. Another type of arrestin, β-arrestin, has also been isolated and shown to specifically inactivate the β-adrenergic receptor [19–21]. Thus, different types of arrestin mediate desensitization of specific receptors in signal transductions. The human arrestin we isolated is retina-specific in expression, homologous to β-arrestin and S-antigen but distinct from them, mapped to the X chromosome (hence named X-arrestin), and localized to the outer and inner segments and inner plexiform region of the retina.

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2. MATERIALS AND METHODS

2.1. *Preparation of retina-enriched cDNA library*

A retina-enriched cDNA library was prepared as previously described [1]. Briefly, mRNA was isolated from the human retina, converted to double-stranded cDNA, subtracted with biotinylated fibroblast cDNA several times using streptavidin in combination with polymerase chain reaction (PCR) amplification, and cloned into pBluescript (Stratagene, La Jolla, CA). Approximately 300 recombinant clones were isolated. The present retina-specific clone was isolated in the initial analysis of 30 clones.

2.2. *Northern blot analysis*

Total human retinal, retinal pigment epithelium, and fibroblast RNA were isolated from tissue or cells using guanidine thiocyanate [22]. Human brain, liver, and lung RNA were obtained from Clonetech (Palo Alto, CA). The RNA was electrophoresed in denaturing agarose gels [23], transferred onto nylon membranes by the Southern blotting method [24], and hybridized with a ^{32}P-labeled DNA probe. The hybridized blots were washed and autoradiographed as described before [25].

2.3. *DNA sequencing*

DNA was sequenced by the dideoxy chain termination method using the Sequenase DNA sequencing kit [26] (United States Biochemical, Cleveland, OH). Sequences were analyzed by the IntelliGenetics (Mountain View, CA) and Genetic Computer Group (Madison, WI) software packages.

2.4. *Sublocalization of X-arrestin on the X chromosome*

High-molecular weight genomic DNA from rodent–human somatic hybrids containing different segments of the human X chromosome

(gift of Dr. T. Mohandas [27]) was digested with *Eco*RI, electrophoresed in 0.8% agarose gels, transferred onto nylon filters by the Southern method [24], and hybridized to ^{32}P-labeled cDNA probes. The hybridized filters were washed and autoradiographed as described [25]. The accuracy of the DNA panel was checked by the hybridization of a known X chromosome marker, TIMP (ATCC, Rockville, MD, [28]), to the blot which showed the correct localization to the Xp region (data not shown).

2.5. In situ hybridization

The whole eye (rat, human) was cut open, fixed in 4% paraformaldehyde, and immersed in 30% sucrose solution overnight. The tissue was frozen, sectioned, fixed again in 4% paraformaldehyde, treated with or without proteinase K, rinsed, and prehybridized in a mixture containing 50% formamide, 10% dextran sulfate, 1 × Denhardt's mixture, and blocking tRNA. The probe was the sense (control) and antisense riboprobes prepared from a truncated X-arrestin Bluescript cDNA clone (1,130–1,314 bp at the 3′ end) by transcription from either the T3 or T7 promoter with incorporation of [^{35}S]ribonucleotides (Stratagene). Hybridization was carried out overnight at 50°C, and the sample was treated with RNase A. The slides were washed with 0.1 × SSC at 55–60°C and subjected to liquid emulsion autoradiography [29–32].

3. RESULTS AND DISCUSSION

A human retina-enriched cDNA library was prepared by repeated subtraction of a retina cDNA population with a biotinylated fibroblast cDNA population followed by amplification of the remaining clones by polymerase chain reaction [1]. Approximately 300 retina-specific or -enriched cDNA clones were obtained, and thirty were initially analyzed. Enrichment for retinal genes was confirmed by the identification of rhodopsin, α-transducin, cGMP-phosphodiesterase γ, and recoverin among the first ten cDNAs analyzed [33].

A cDNA clone was identified which showed a highly retina-specific pattern of expression on Northern analysis of RNA from human liver, lung, brain, fibroblast, retinal pigment epithelium, and neuroretina (Fig. 1). A single specie of 1.35 kb was observed. Sequence analysis of the clone revealed a nearly full-length 1,314 bp cDNA with a polyadenylation signal at position 1,269 and a poly(A) sequence at the 3′ end (Fig. 2). An open reading frame began with an ATG codon at position 52, with a good consensus sequence for translation initiation codon [34], and ended with a termination codon at position 1,216, resulting in a protein of 388 amino acid residues and a calculated molecular mass of 42,864.

Comparison of the protein sequence with those in the Protein Information Resource sequence databank by the FastDB program (Intelligenetics Inc.) showed it to be most similar to arrestin, a protein involved in receptor-mediated homologous desensitization of retinal photoreceptors [13,14], and β-adrenergic receptors [19]. The highest homology was with bovine β-arrestin [19] at 58%, followed by rat β-arrestin 1 [20] at 57%, human thyroid arrestin (putative human β-arrestin) [21], and rat β-arrestin 2 [20] at 52%, bovine retinal and rat pineal arrestin (S-antigen) [15,16] at 50%, human retinal arrestin (S-antigen) [5] at 49%, and *Drosophila miranda*

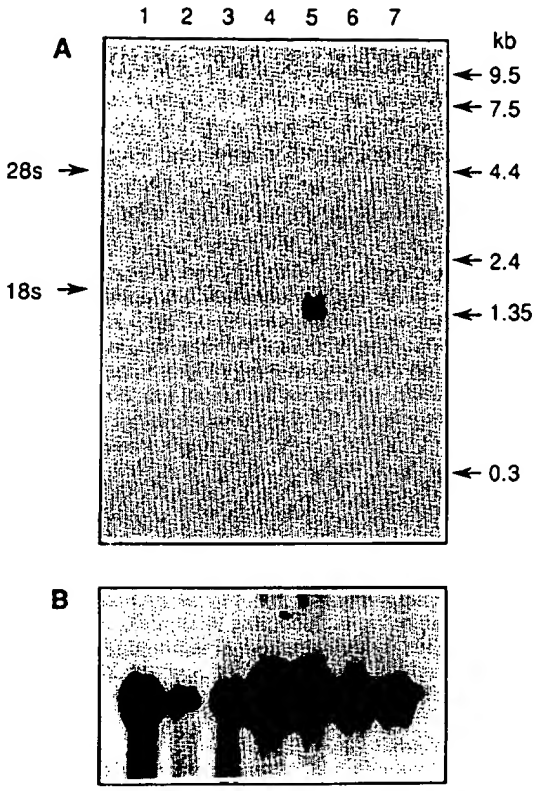

Fig. 1. Northern blot analysis of human mRNA with the retinal cDNA probe. RNA from different human tissues are present in lanes 1–7: 1, lung; 2, liver; 3, brain; 4, retinal pigment epithelium; 5, neuroretina; 6 and 7, skin fibroblast. (A) Hybridized with ^{32}P-labeled cDNA clone; arrow points to the transcript of approximately 1.35 kb seen only in the retina. (B) Hybridized with the human β-actin probe to check on the quantity and quality of RNA present in each lane. Positions of the 28 S and 18 S ribosomal RNA and RNA standards are shown.

arrestin [35] at 37%. Despite the highest homology with the β-arrestins, the new arrestin (X-arrestin) seemed to be distinct from the β-arrestins in that the homology between X-arrestin and the β-arrestins (52–58%) was significantly lower than the homology among the human, rat, and bovine homologues of β-arrestin (74–99%) [20,21]. X-Arrestin was also clearly distinct from the original retinal arrestins (S-antigen) since it was only 49–50% homologous to them, while the homology among the retinal arrestins is over 80% [5,15,16]. Thus, the bovine β-arrestin, human thyroid arrestin (putative β), bovine and human retinal arrestin (S-antigen), and X-arrestin sequences were multiply aligned by the GENALIGN program (Intelligenetics Inc.) in order to closely examine the nature of the homologies (Fig. 3).

The homology among these arrestins existed throughout the sequence except for the carboxy-terminals. Divergence of the carboxy-terminal sequence in the arrestins has been noted previously [19,20]. Forty to sixty residues at the carboxy-termini of arrestins showed divergence, with the β-arrestins and the retinal arrestins

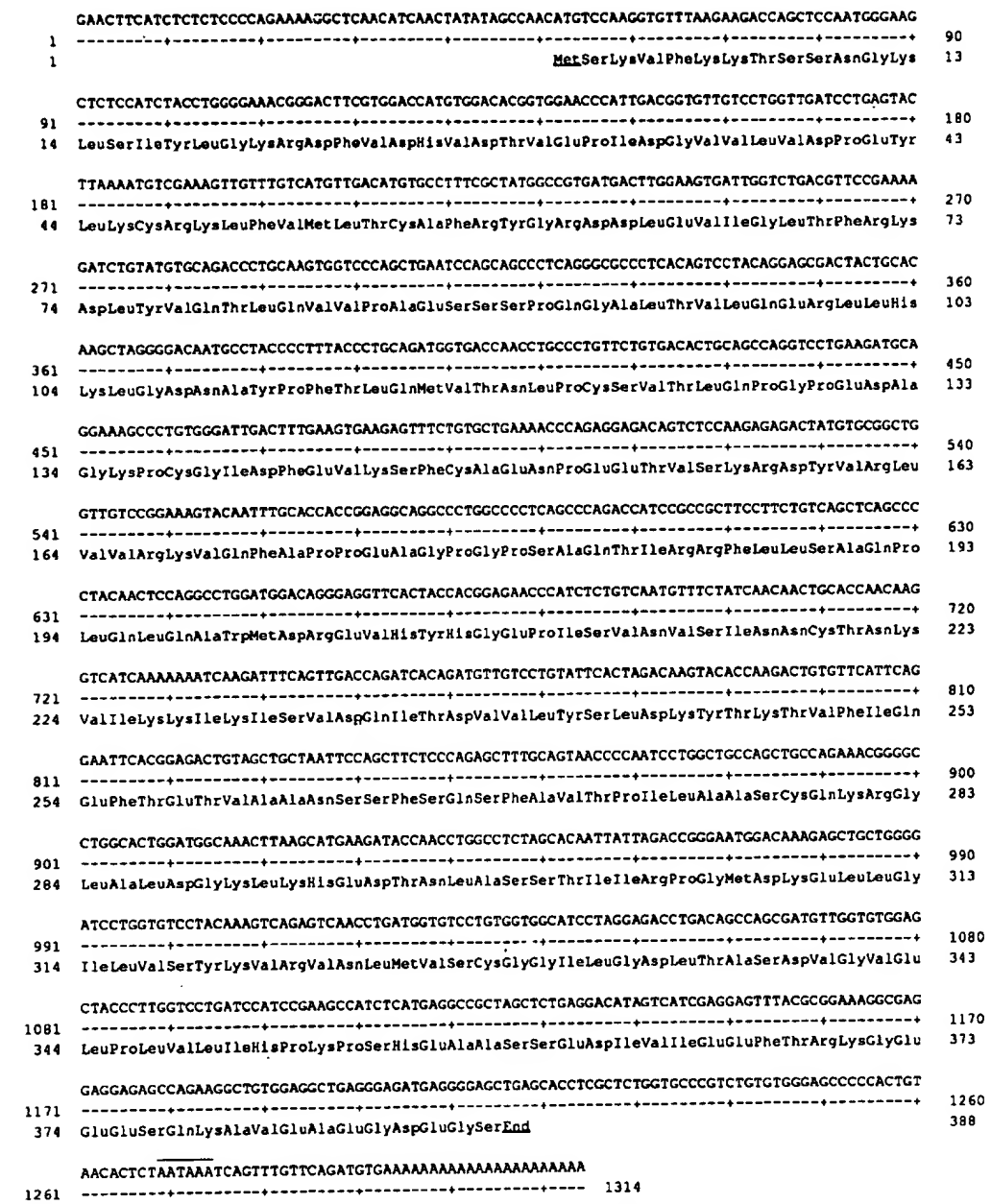

Fig. 2. Nucleotide and derived protein sequence of the retinal cDNA. The putative initiation methionine and termination codons are underlined. The translation yields a 388 amino acid protein. The polyadenylation signal at nucleotide 1,269 is overlined.

(S-antigen) losing homology at around residue 330, X-arrestin losing homology at around residue 353, and the two β-arrestins (bovine and human thyroid) and the two retinal arrestins (bovine and human S-antigen) retaining homology relatively well to the carboxy termini. Thus, the uniqueness of X-arrestin's carboxy-terminal region seemed to support its distinctness from the other arrestins.

Partial sequence similarities between arrestin and transducin α, a guanine nucleotide-binding, signal-coupling protein [36–38], have been demonstrated, and they are designated in Fig. 3. These include three phosphoryl binding sites and the pertussis toxin ADP-ribosylation site described in retinal arrestin (S-antigen) [5,39], and five regions similar to transducin α described in bovine β-arrestin [19]. These sequence similarities support the hypothesis that arrestin mimics transducin and interacts directly with the sensory receptor to result in homologous desensitization [5,13,14]. Binding of retinal arrestin to phosphorylated photoreceptor rhodopsin and β-arrestin to phosphorylated β-adrenergic receptor with subsequent desensitization have been shown [14,19]. X-

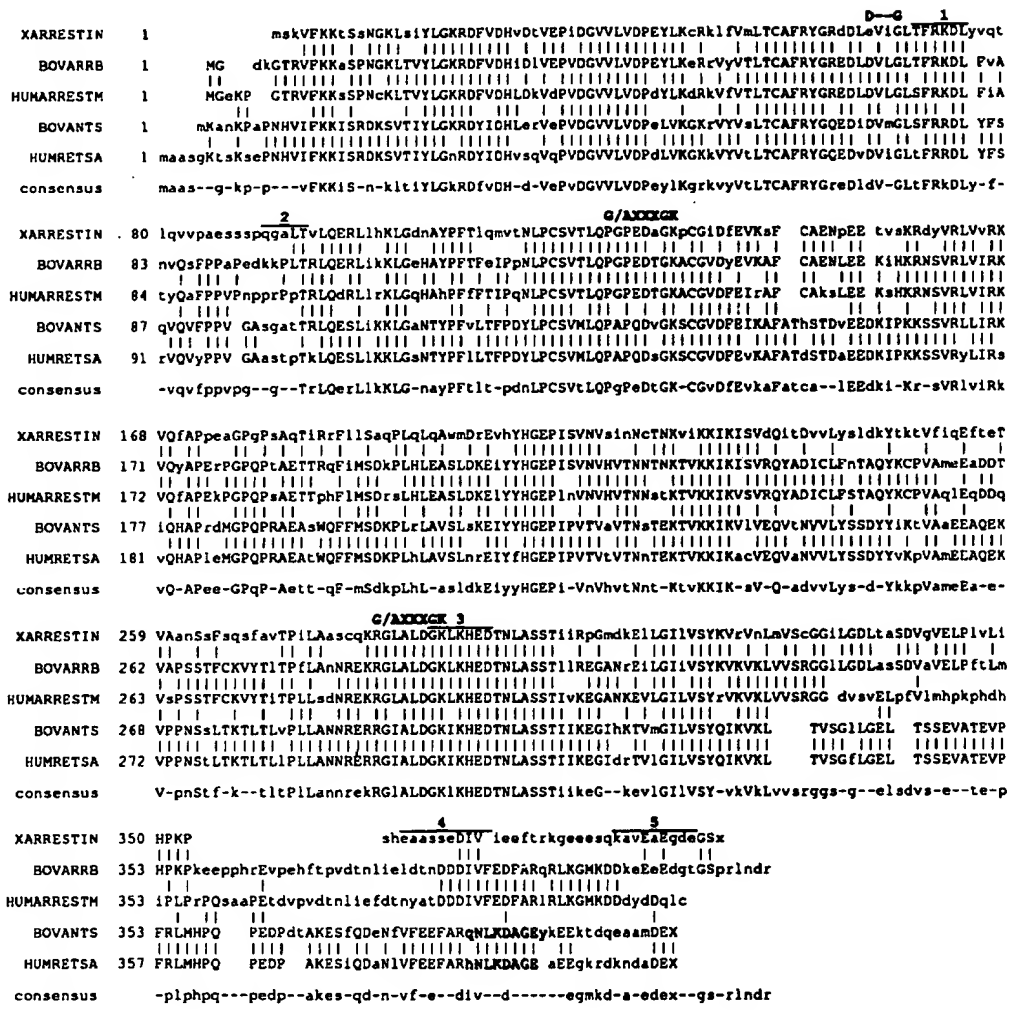

Fig. 3. Alignment of multiple arrestin sequences. The protein sequences of the new X-arrestin (XARRESTIN), bovine β-arrestin (BOVARRB), human thyroid arrestin (HUMARRESTM), bovine retinal arrestin (BOVANTS, S-antigen), and human retinal arrestin (HUMRETSA, S-antigen) were multiply aligned by the GENALIGN computer program (IntelliGenetics, Inc.). D--G and G/AXXXGK designate three regions showing homology to GTP phosphoryl binding sites, and the shadowed residues in the BOVANTS and HUMRETSA sequences are homologous to the pertussis toxin ADP-ribosylation site [5,39]. The regions designated 1–5 have been shown to be similar to transducin α in bovine β-arrestin [19].

Arrestin contains the three phosphoryl binding sites and regions 1 and 3 of the transducin α-like sequences. These sequences are common to all of the arrestins being compared, whereas regions 2 and 5 are present only in bovine β-arrestin and region 4 is common to bovine β-arrestin and the putative human homologue, thyroid arrestin. Notably, the ADP-ribosylation site is not present in X-arrestin or the bovine and human (thyroid) β-arrestins.

Chromosomal mapping of the X-arrestin gene by hybridization to panels of DNA from somatic cell hybrids containing specific human chromosomes (Oncor Inc., Gaithersburg, MD) (data not shown) and specific parts of the human X chromosome (Fig. 4) [27] localized the gene to Xcen-Xq21. Thus, X-arrestin joins the ranks of other retinal genes that have been localized to X, including the red and green opsins, and the genes for choroideremia and Norrie disease [2,40–42]. Interestingly, a *Drosophila* arrestin in *D. miranda*, to which X-arrestin shows the highest homology (37%) among fly arrestins, has also been mapped to X [35]. This arrestin is a homologue of that described by LeVine et al. (Arr2) [43] and Yamada et al. [17], and is distinct from Arr1, a homologue of S-antigen [43]. An interesting functional study of Arr2 has been published recently [44], and its relevance to X-arrestin will be discussed later.

In situ hybridization analysis of X-arrestin using the rat and human retina demonstrated expression of this gene in the inner and outer segment and inner plexiform regions (Fig. 5). In view of the close homology among all the arrestins, the analysis was performed with a partial probe from the 3′ end (1,130–1,314 bp) of the cDNA

representing the unique part of the X-arrestin sequence. A similar pattern of reaction in the photoreceptor cells, inner plexiform layer, and biopolar cells has been shown in a newt with antibodies against S- antigen [45]. The authors suggested that the reaction in the inner plexiform and bipolar cells may be due to arrestin related to β-adrenergic or similar chemical signal receptors.

The presence of up to four different arrestins has been reported [46]. The precise identity and function of X-arrestin needs to be determined. Which receptor signal transduction does it regulate? Its distinctness from S-antigen and β-adrenergic arrestin appears to be clear on the basis of the sequence differences and the uniqueness of the carboxy terminal region. It does not even appear to be a homologue of the third type of arrestin, represented by the rat β-arrestin 2 [20] since its homology to β-arrestin 2 is 52%, similar to β-arrestin 1 at 57%. Despite its slightly higher homology to β-arrestin, its expression pattern (retina-specific) is more like that of S-antigen than that of β-arrestin, which is expressed in multiple tissues [19]. Some of the in situ hybridization observed in the retinal inner and outer segments may represent cross-hybridization with S-antigen message (despite the use of a unique X-arrestin probe), but much of it most likely represents X-arrestin expression.

S-Antigen has been postulated to mimic transducin α and interact directly with photoactivated rhodopsin [5,13,14]. In this regard, the carboxy-terminal region including the ADP-ribosylation site has been considered to be important [47,48]. The dissimilarity of the carboxy-termini, including the lack of the ADP-ribosylation site in X-arrestin compared to S-antigen, appears to argue against identical functions for both, i.e. desensitization of rhodopsin signal transduction. Considering the sequence conservation between rhodopsin and color opsins [2], the involvement of X-arrestin in cone transduction also appears unlikely on the same grounds. The abundant pattern of in situ hybridization in the inner and outer segments also does not appear to support the presence of X-arrestin only in the cones. A recent report of *Drosophila* arrestin 2, however, is interesting in that, in addition to arrestin 1, which has been considered to be the homologue of S-antigen [17,18], it was also shown to be involved in the inactivation of rhodopsin [44]. The distribution of arrestin 2 in the fly retina was identical to that of arrestin 1, somewhat similar to the localization of X-arrestin to the inner and outer segments where S-antigen is present. Arrestin 2 is the homologue of *D. miranda* arrestin (98% homology), which showed the highest homology to X-arrestin among the fly arrestins and which also mapped to the X chromosomes [35]. Despite the species difference, the human X-arrestin may be functionally similar to the *Drosophila* arrestin 2, and may also be involved in inactivation of rhodopsin.

Alternatively, X-arrestin may play a role in an as yet undefined retina-specific signal transduction. In this respect, its expression in the inner plexiform region by in situ hybridization is especially interesting. β-Adrenergic transduction might be a possibility in the inner plexiform except we have shown that X-arrestin is distinct from β-arrestin. Cross-hybridization with β-arrestin message is a possibility, but use of the unique probe should have minimized it. The inner plexiform is where the bipolar, amacrine, and ganglion cells synapse, a site presumably of a variety of chemical signal transductions. X-Arrestin may be involved in one of these synaptic signal transductions that is highly retina-specific.

Recently, new α-G-proteins have been isolated that

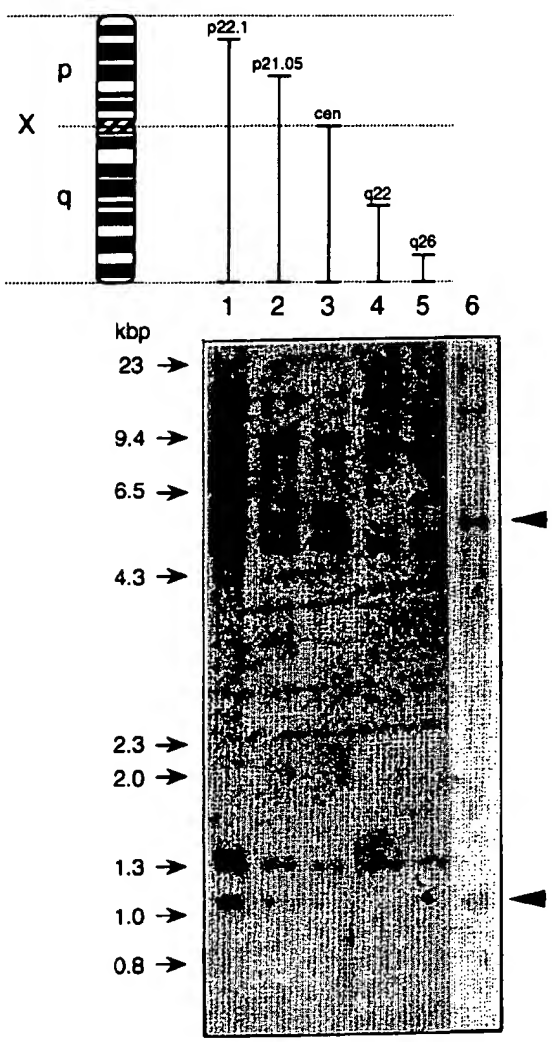

Fig. 4. Sublocalization of X-arrestin on the X chromosome. The hybridization of the X-arrestin probe to *Eco*RI-digested genomic DNA from rodent–human somatic hybrids containing different segments of the human X chromosome (gift of Dr. T. Mohandas) [27] is shown. The X segment contained in each hybrid is schematically shown at the top. The arrowheads point to the X-arrestin gene bands visible in some of the hybrid DNA and normal human genomic DNA (lane 6). The DNA size markers are *Hin*dIII-digested λ DNA.

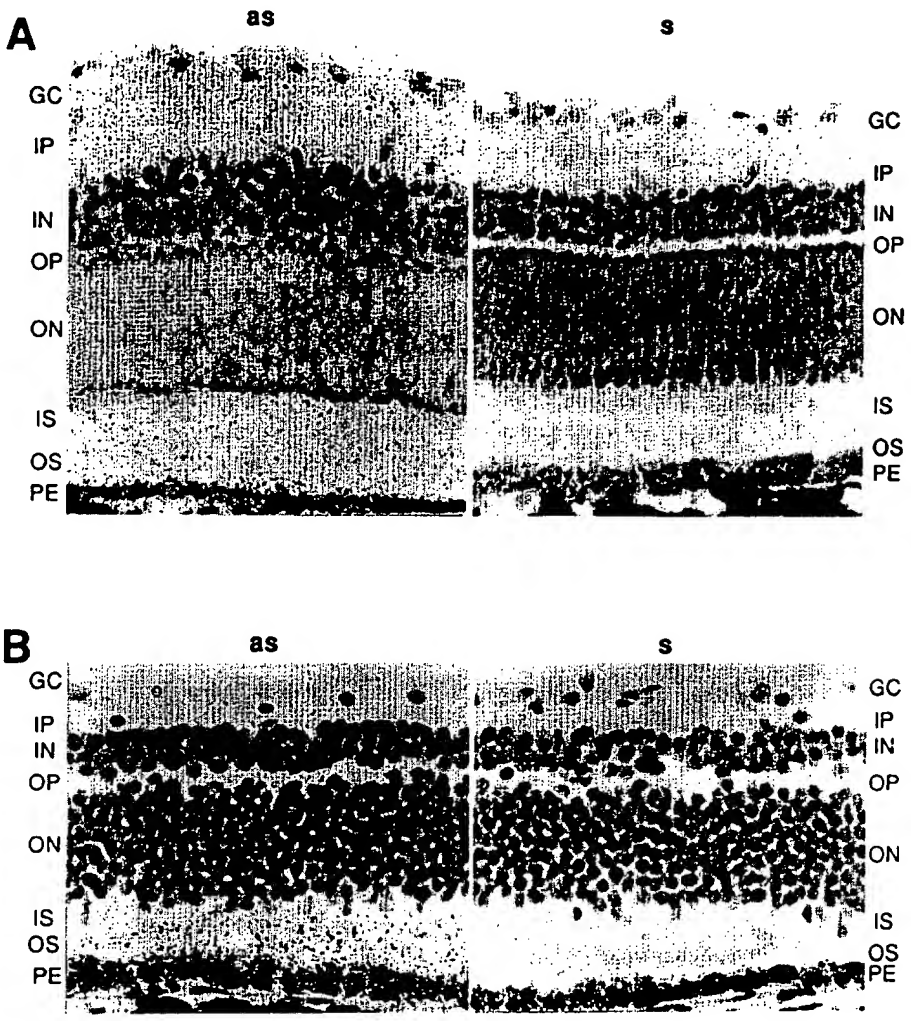

Fig. 5. In situ hybridization of X-arrestin in the rat and human retina. The results of liquid emulsion autoradiography of the retina sections after hybridization with the X-arrestin sense or antisense riboprobes are shown. (A) Rat retina; (B) human retina. as, antisense; s, sense; GC, ganglion cell layer; IP, inner plexiform; IN, inner nuclear layer; OP, outer plexiform; ON, outer nuclear layer; IS, inner segment; OS, outer segment; PE, pigment epithelium.

lack the carboxyl ADP-ribosylation site and are insensitive to pertussis toxin [49,50]. These G-proteins are suggested to play a role in signal transductions involving receptor-mediated activation of phospholipase C, as is the case in invertebrate phototransduction [50]. Phospholipase C has been demonstrated in bovine rod outer segments (ROS) [51], and its activation by light has been shown in several species [51–53]. In fact, activation of bovine ROS phospholipase C by arrestin (assumed to be S-antigen) was recently reported [54]. In view of the absence of pertussis toxin ADP-ribosylation site in X-arrestin, it is interesting to speculate that X-arrestin may be involved in such a system. If true, X-arrestin may indeed be very similar functionally to the *Drosophila* arrestin 2 [44]. In vitro expression of the gene product and biochemical testing of such functions as quenching of opsin-mediated and β-adrenergic receptor-mediated transduction, and activation of phospholipase C should help in identifying X-arrestin.

Note added in proof

Recent personal communication has indicated that Dr. C. Craft and colleagues may have isolated a similar arrestin.

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